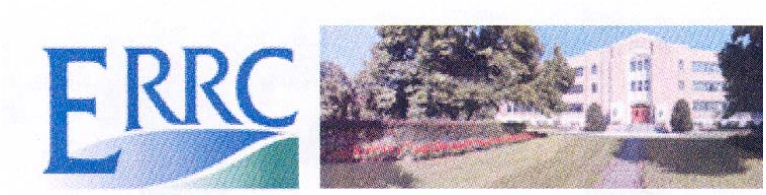

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Research Note

Thermal Inactivation of *Escherichia coli* O157:H7 in Ground Beef Supplemented with Sodium Lactate†

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ABSTRACT

A study was conducted to investigate the antimicrobial effect of sodium lactate (NaL) (0, 1.5, 3.0, and 4.5%) on the survival of *Escherichia coli* O157:H7 in 93% lean ground beef. Samples inoculated with a mixture of four strains of *E. coli* O157:H7 (10^7 to 10^8 CFU/g) were subjected to immersion heating in a water bath stabilized at 55, 57.5, 60, 62.5, or 65°C. Results of statistical analysis indicated that the heating temperature was the only factor affecting the decimal reduction times (*D*-values) of *E. coli* O157:H7 in 93% lean ground beef. The change in temperature required to change the *D*-value (the *z*-value) was determined as 7.6°C. The thermal resistance of this organism was neither affected by the addition of NaL nor by the interactions between NaL and temperature. Adding NaL to ground beef to reduce the thermal resistance of *E. coli* O157:H7 is therefore not recommended.

Contamination of ground beef with *Escherichia coli* O157:H7 is a serious health hazard and concern for both consumers and food processors. Outbreaks of *E. coli* O157:H7 associated with contaminated ground beef have caused hospitalizations and fatalities since 1982 *(2, 7)*. Major recalls of raw beef hamburgers have been issued in recent years by the U.S. Department of Agriculture to protect the public health.

Sodium lactate (NaL) had been originally approved for use in the meat and poultry industry as humectants and flavor enhancers. Levels at 2.0% or above can be added to hams, frankfurters, and similar products to increase cooking yields and water-holding capacity. NaL also is a known antimicrobial agent that inhibits many spoilage and pathogenic microorganism commonly found in cooked meat and poultry products *(3)*. Therefore, NaL has been widely used in the food industry to extend the shelf life of cured meat products, fish, and uncured meat *(4, 5)*.

Although NaL is an effective antimicrobial agent, there appears to be a lack of information in the published scientific literature regarding the efficacy of NaL on the thermal resistance of *E. coli* O157:H7 in ground beef. Accordingly, the objective of this study was to use the general antimicrobial capability of NaL and investigate the combined effect of NaL and temperature on the inactivation of *E. coli* O157:H7 in ground beef during thermal processing. Our hypothesis was that NaL could significantly decrease the thermal resistance of *E. coli* O157:H7 in ground beef and consequently reduce the cooking requirements for raw ground beef patties.

MATERIALS AND METHODS

Ground beef. Ground beef (93% lean by label) was purchased from a local grocery store. The meat was frozen (−18°C) until use (<2 months). Prior to experimentation, 50 g of frozen ground meat in a plastic bag was taken out of the freezer, immersed in a 2-liter beaker, and thawed with cold running water.

Organisms. Four strains (EDL-931, 45753-35, C1-9218, and 933) of *E. coli* O157:H7 *(6)* were obtained from the culture collection of the Microbial Food Safety Research Unit in the Eastern Regional Research Center of the U.S. Department of Agriculture, Agricultural Research Service (Wyndmoor, Pa.). The bacterial strains were propagated and maintained, and their purity was periodically checked with Vitek Test Cards (Gram-Negative Identification Card Plus, or GNI+) using the Vitek Automated Microbiology System (Model Vitek 60, bioMerieux, Inc., Hazelwood, Mo.).

Inoculum preparation. One loopful of each culture was transferred to 10 ml of brain heart infusion broth (Difco Laboratories, Detroit, Mich.) and incubated for 24 h at 37°C. Each stationary-phase culture was centrifuged (2,400 × *g*) at 4°C for 15 min and washed twice with 10 ml of sterile 0.1% peptone water (wt/vol). Bacterial cell pellets of each strain were resuspended in 10 ml of 0.1% peptone water. A 5-ml aliquot of each strain was combined and mixed to prepare a four-strain bacterial cocktail.

Sample preparation, inoculation, and storage. The bacterial cocktail (10 ml) prepared previously was aseptically added to thawed ground beef (50 g) in a sterile plastic bag (19 by 30 cm, Spiral Biotech, Bethesda, Md.) and mixed at high speed for 10 min in a stomacher (Model 400 Lab-Blender, Tekmar, Cincinnati, Ohio). Then, the inoculated meat was equally divided into four portions in sterile plastic filter bags (12 by 19 cm, Model BagPage

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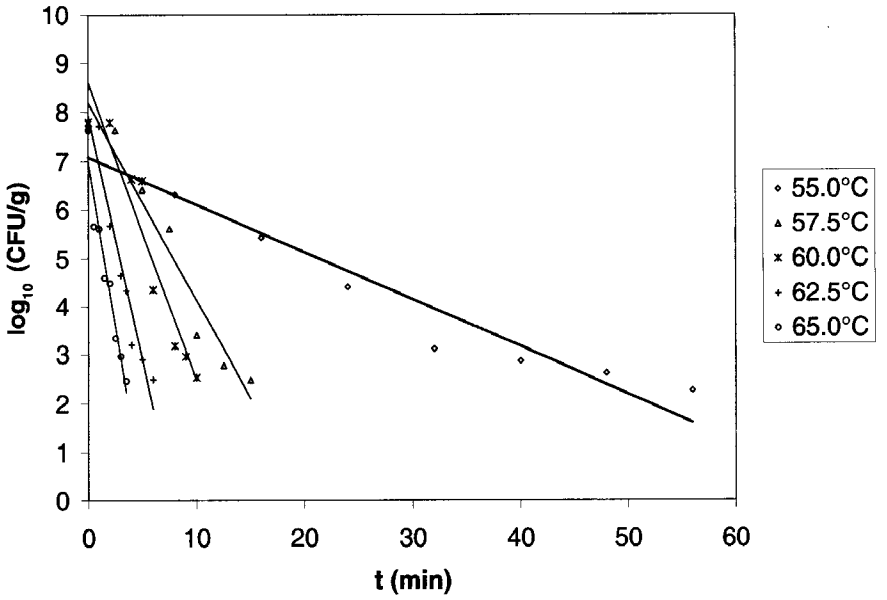

FIGURE 1. *Representative survivor curves of the four-strain cocktail of* E. coli *O157:H7 in ground beef heated at different temperatures.*

BP 100, Topac Inc., Hingham, Mass.). Different levels (0, 1.5, 3.0, and 4.5% [wt/wt]) of NaL (L-7900, 60% [wt/wt] syrup, Sigma Chemical Co., St. Louis, Mo.) were added to each meat portion. The mixture was pummeled at the maximum speed for 6 min in a MiniMix stomacher (Model BagMixer 100 W, Interscience Co., St. Nom-La Breteche, France). After mixing, ground beef samples were divided into 5- ± 0.02-g portions and packaged in plastic filter bags (Model BagPage BP 100, Topac). The meat in each plastic bag was pressed against a flat surface so that the meat was evenly distributed. The thickness of meat was approximately 1 to 1.5 mm. Each plastic bag was vacuumed to evacuate air and sealed at a final vacuum level of 15 mm Hg (2,000 Pa). Removing the air from the plastic bags containing the ground beef samples ensured tight contact between the ground beef and the filter bags, thus eliminating the insulation effect caused by air during thermal treatment.

Thermal inactivation. Samples were fully immersed in a temperature-controlled circulating water bath (Model ESRB-7, Techne Inc., Princeton, N.J.). Each sample was separated approximately 1 cm from one another to ensure uniform heating. Temperatures of the water bath were stabilized at 55.0, 57.5, 60.0, 62.5, and 65.0 ± 0.01°C. Come-up times were measured by inserting two thermocouples (Type J, AWG 36; Omega Engineering, Inc., Stamford, Conn.) into two separate sample bags. The thermocouples, imbedded in the meat, were also vacuum sealed with the samples. The internal temperatures were monitored at 1-s intervals by datalogging software (Labview 6.0, National Instruments Corp., Austin, Tex.) and a data acquisition board (Model NI 4351, National Instruments). The come-up times ranged between 8 and 10 s.

At different time intervals, depending on temperature, samples were removed from the water bath, immediately submerged in an ice-water bath, and analyzed within 30 min. Each temperature and NaL combination was replicated twice.

Enumeration of surviving bacteria. Sterile 0.1% peptone water (10 ml) was added to each sample and pummeled for 6 min at the maximum speed in a MiniMix stomacher (Model BagMixer 100 W, Interscience). Serial dilutions were made and surface plated onto tryptic soy agar (TSA; Difco) plates. After a 120-min resuscitation at room temperature to allow for the recovery of heat-damaged cells *(9)*, the TSA plates were overlaid with approximately 10 ml of MacConkey sorbitol agar (Difco) that was previously tempered to 47°C. After solidification, sample plates were incubated at 37 ± 1°C for 30 h. Typical *E. coli* O157:H7 colonies were counted after incubation. An average of three plate readings per sampling point were used to represent the number of bacteria surviving after each heat treatment. The bacterial counts were converted to $\log_{10}$ (CFU/g) and used to calculate decimal reduction times (*D*-values).

Linear model. The *D*-value of *E. coli* O157:H7 at a constant temperature was determined by the linear regression between the experimental survival data (*C*) and the heating time (equation 1). The change in temperature required to change the *D*-value (the *z*-value) of the four-strain cocktail of *E. coli* O157:H7 in 93% lean ground beef was obtained by the linear regression between $\log_{10}(D)$ and temperature (*T*), as expressed in equation 2.

$$\log_{10}(C) = \log_{10}(C_0) - \frac{t}{D} \quad (1)$$

$$\log_{10}(D) = \log_{10}(D_0) - \frac{T}{z} \quad (2)$$

Statistical analyses. SAS version 8 *(10)* was used to compare the effect of different treatments on the thermal inactivation

TABLE 1. *Statistical analysis of the effect of temperature and NaL on the* D*-values of* Escherichia coli *O157:H7 in ground beef*[a]

Source	df	Type I SS	Mean square	F value	Pr > F
Temperature	4	599.63	149.9	954.6	<0.0001
NaL	3	0.07	0.02	0.1	0.93
Temperature × NaL	12	0.05	0.004	0.03	1.00

[a] df, degrees of freedom; SS, sum of squares; Pr, probability.

TABLE 2. *D-values (minutes) of* Escherichia coli *O157:H7 in 93% lean ground beef at 55 to 65°C*

	D-values of *E. coli* O157:H7 with NaL levels (%) of:			
Temp (°C)	0.0	1.5	3.0	4.5
55.0	11.13 A ± 0.85[a]	11.16 A ± 0.88	10.91 A ± 0.98	11.02 A ± 0.75
57.5	2.64 B ± 0.13	2.71 B ± 0.23	2.55 B ± 0.11	2.61 B ± 0.11
60.0	1.71 C ± 0.01	1.69 C ± 0.05	1.65 C ± 0.02	1.72 C ± 0.05
62.5	1.03 DE ± 0.04	1.01 DE ± 0.01	0.97 DE ± 0.04	1.03 DE ± 0.04
65.0	0.75 E ± 0.01	0.73 E ± 0.04	0.71 E ± 0.05	0.73 E ± 0.04

[a] Mean ± standard deviation. Means with the same letter are not statistically different ($P < 0.05$).

of *E. coli* O157:H7 in 93% lean ground beef. The general linear models procedure was used to analyze the effect of temperature and NaL on the *D*-values of *E. coli* O157:H7. The Tukey's studentized range test at $\alpha = 0.05$ was conducted to compare the effect of NaL and heating temperature on the means of the *D*-values of *E. coli* O157:H7.

RESULTS AND DISCUSSION

The average initial inoculum of *E. coli* O157:H7 in the samples was 7.69 $\log_{10}$ (CFU/g) with a very small standard error (0.01), indicating the beef samples were homogeneously inoculated. Figure 1 shows typical thermal inactivation (or survival) curves observed in this study. From the experimental observation, it was evident that the first-order thermal inactivation kinetics could be used to fit all the survival curves (equation 1) and calculate the *D*-values.

Table 1 lists the results of statistical analysis of the effect of temperature and NaL on the *D*-values of *E. coli* O157:H7 in 93% lean ground beef. Evidently, the *D*-values of *E. coli* O157:H7 were significantly affected by temperature ($P < 0.0001$) but were not influenced by NaL ($P = 0.93$) nor by the interactions between NaL and the heating temperature ($P = 1.00$). On the basis of the results from the statistical analysis, the hypothesis that NaL could significantly reduce the thermal resistance of *E. coli* O157:H7 in ground beef was therefore rejected, and the null hypothesis that NaL had no effect on thermal resistance of this organism in ground beef was thus accepted.

Table 2 lists the mean *D*-values of *E. coli* O157:H7 in 93% lean ground beef at temperatures between 55 and 65°C. Since NaL showed no effect on the *D*-values of *E. coli* O157:H7 in 93% lean ground beef, all the *D*-values of the same heating temperature could be combined to construct a $\log_{10}(D) - T$ plot (Fig. 2). According to the Tukey's studentized range test, no statistical difference was found between the means of the *D*-values of *E. coli* O157:H7 at 62.5 and 65°C, indicating the *D*-values obtained at 65°C were probably unreliable and should be excluded in determining the *z*-value of this organism. The mean *z*-value, estimated by the linear regression between $\log_{10}(D)$ and *T*, was 7.6°C with a standard error of 0.4°C. This value is slightly higher than the value (6°C) reported by Juneja et al. *(6)*.

Although NaL can inhibit the growth of a spectrum of microorganisms, including *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Pseudomonas fragi, Yersinia enterocolitica, Listeria monocytogenes,* and *Clostridium sporogenes (5, 11),* De Wit and Rombouts *(3)* observed that it had no inhibitory effect against *E. coli* at a concentration as high as 5% in a neutral culture media. NaL is primarily used in cooked meat products such as hams and sausages to extend cold storage. It is very effective in inhibiting the predominant spoilage flora, such as psychrotrophic lactic acid bacteria. A number of reports in the literature support this observation. Potassium lactate at 2% could be added

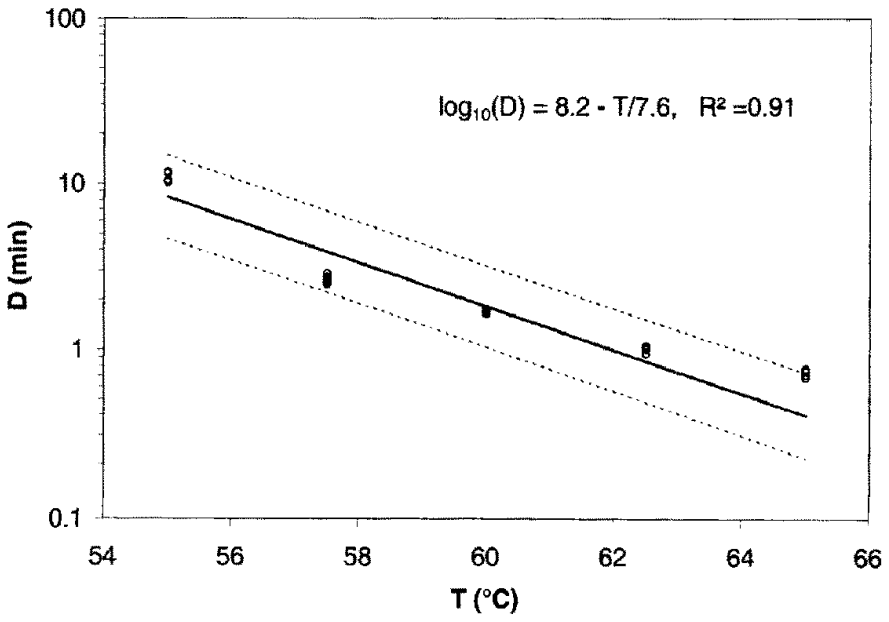

FIGURE 2. *Effect of temperature on the* D*-values of* E. coli *O157:H7 in cooked ground beef. Dotted lines represent upper and lower prediction limits at a 95% confidence level.*

to a low-fat fresh pork sausage to lower bacterial counts during refrigerated storage *(1)*. The microbial activity could be effectively decreased with the addition of NaL to vacuum-packaged ground beef at 4°C *(8)*.

According to De Wit and Rombouts *(3)*, the antimicrobial effect of NaL was not attributed to its ability to lower water activity, but rather, to the ability of the organisms to withstand this compound. *E. coli* O157:H7 is probably capable of tolerating NaL and, consequently, insensitive to this compound at normal growth temperatures. This study demonstrates that *E. coli* O157:H7 could also tolerate NaL up to 4.5% in ground beef, even in elevated temperature conditions. Therefore, adding NaL to ground beef to reduce the thermal resistance of this organism in raw ground beef patties is not recommended.

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